Observations on the Urine in Chronic Disease of the Pancreas.

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Modern researches on the physiology of the pancreas have shown that it plays a much more important part in digestion than had formerly been supposed, and also indicated that it exerts a very considerable influence on the internal metabolism of the body. The present investigations were commenced early in 1901, at the suggestion of Mr. Mayo Robson, with the object of throwing further light on the nature of these metabolic processes and discovering, if possible, some more reliable means of diagnosing diseases of the pancreas than is usually afforded by the clinical signs.

The condition of the blood in patients suffering from diseases of the pancreas was first investigated, and subsequently attention was devoted to the urine. The clinical bearing of the results of these observations has been dealt with in my Arris and Gale Lecture, and Mr. Robson's Hunterian Lectures, delivered at the Royal College of Surgeons in 1904,* and also in a paper I read before the Royal Medical and Chirurgical Society in 1906.†

In the course of a series of experiments designed to discover whether glycerine, the soluble product of the disseminated fat necrosis met with in pancreatitis, or some derivative of it, was excreted by the kidneys, I found that if the urine of a person suffering from an inflammatory affection of the pancreas were boiled with hydrochloric acid, the excess of acid neutralised with lead carbonate, and the freed glycuronic acid precipitated out of the acid solution with tri-basic lead acetate, treatment of the filtrate with phenylhydrazin yielded a crystalline product which appeared to vary in amount with the stage and intensity of the disease, while normal urines and specimens from patients suffering from diseases in which there was no reason to think that the pancreas was involved gave no reaction. In this communication I propose to describe a series of experiments I have conducted into the nature of this product, and also the results of animal experiments designed to discover whether the reaction depended upon changes in the pancreas itself or was due to alterations in the other tissues of the body brought about by a disturbance of its metabolic functions.

For the purpose of the former investigations 4 litres of urine from

* 'Lancet,' March 19, 26, April 2, 1904.

† 'Roy. Med. Chi. Soc. Trans.,' 1906.

each of eight patients, under the care of Mr. Mayo Robson, were collected and separately examined, but as the methods employed and the results obtained were similar in all it will not be necessary to deal with them individually. The diagnosis of "pancreatitis" in these cases was based upon the clinical symptoms, analyses of the fæces, and the condition of the pancreas found at operation, while in one instance it was confirmed by histological examination of the gland after death. In five the pancreatitis was associated with, and was probably dependent upon, the presence of gall-stones in the common bile duct; two had had symptoms of "indigestion" for several years, which in one was believed to have originated in an attack of typhoid fever, and in the other had come on insidiously, but was associated with evidence pointing to excessive putrefactive changes in the contents of the intestine and an abnormal intestinal flora. In one there was sub-acute pancreatitis with disseminated fat necrosis, and an impacted gall-stone in the ampulla of Vater; *post-mortem* examination in this case showed an abscess in the tail of the pancreas and staining of the walls of the duct of Wirsung, with bile for a distance of about 3 inches from its junction with the common bile duct. Sections of the gland on microscopical examination showed a considerable over-growth of the interlobular connective tissue with some small round-celled inflammatory infiltration and vacuolisation of the acinar cells, which stained feebly.

The urine from these patients, which had been preserved with chloroform, was filtered to remove suspended matter, and to the 4 litres were added 400 c.c. of hydrochloric acid (sp. gr. 1·16). The mixture was placed in a flask with a funnel in the mouth to act as a condenser, and the flask heated on a sand-bath until the contents boiled. After being kept gently boiling for 10 minutes the flask was cooled in running water, the urine made up to its original volume with distilled water, and the excess of acid neutralised by slowly adding 1600 grammes of lead carbonate. On the completion of the reaction it was filtered through a moist filter-paper, and the acid filtrate shaken with 1600 grammes of tri-basic lead acetate. The filtrate from this was made alkaline with ammonia, and the resulting precipitate washed with distilled water until the washings no longer acted upon red litmus paper. The precipitate was then suspended in 200 c.c. of distilled water, made faintly acid with hydrochloric acid, treated with a stream of sulphuretted hydrogen, and the precipitated lead sulphide removed by filtration. The clear filtrate was gently warmed on a water-bath and, when free from sulphuretted hydrogen, was again well shaken with 30 grammes of tri-basic lead acetate, filtered, and the filtrate made alkaline with ammonia. The precipitate that formed, after being washed

with distilled water until the washings were neutral to litmus, was suspended in 100 c.c. of distilled water, treated with sulphuretted hydrogen, filtered, and the filtrate gently warmed to expel the excess of gas. It was then cooled and again filtered. The resulting clear solution was then examined as follows :—

A. The fluid gave all the usual carbohydrate reactions: Molisch's test was positive; heating with concentrated sulphuric acid caused it to quickly blacken; Moore's test gave a brown coloration; Tollen's ammoniacal silver nitrate solution showed a black precipitate at room temperature in a few minutes; alkaline solutions of copper sulphate and bismuth were readily reduced, but a solution of copper acetate in acetic acid (Barford's test) was not reduced, showing that the carbohydrate was not dextrose. The presence of free aldehyde was excluded by the absence of any reaction with a solution of rosaniline decolorised by sulphur dioxide.

B. Examined with the polariscope the fluid was found to be optically inactive.

C. Fifty cubic centimetres of the solution were mixed with 2 grammes of phenylhydrazin hydrochlorate and 6 grammes of sodium acetate, and heated in a water-bath at 100° C. for two hours. On cooling, a dense flocculent light yellow precipitate appeared. Microscopically, this was found to consist of long, flexible, hair-like crystals, which, on being irrigated with 33-per-cent. sulphuric acid, disappeared within a few seconds of the acid reaching them, suggesting that they were either a pentosazone or maltosazone.* The precipitate was filtered off, well washed with cold distilled water, and recrystallised from hot 10-per-cent. alcohol three times. It was then dried in a hot-water oven, cooled in a desiccator, and examined.

(1) The crystals were found to be soluble in water at 60° to 70° C., like a pentosazone.†

(2) A melting-point of 178° to 180° C. was obtained with seven of the specimens; one, that obtained from the patient with sub-acute pancreatitis, softened at 160° C., but did not completely melt until the temperature reached 178° to 180° C.

(3) Estimation of the nitrogen-content by Dumas's method gave 17·02 per cent., 17·01 per cent., 16·62 per cent., 17·00 per cent., 16·40 per cent., 16·86 per cent., 17·11 per cent., 17·08 per cent. of nitrogen, readings which fall within the limits of experimental error of the calculated 17·07 per cent. of nitrogen for pentosazone.

* 'Roy. Med. Chi. Soc. Trans.,' vol. 88, p. 265.

† Neubauer and Vogel, 'Analyse des Harns,' 1898, p. 88.

D. The aniline acetate test, when applied to the original solution,* gave a uniform bright red colour. The phloroglucin test was also positive, showing a bright red colour at the boiling-point and forming a dark purple precipitate after boiling for about one minute. Spectroscopical examination of a solution of this precipitate in 93-per-cent. alcohol revealed an absorption band in the green, to the right of D. Tollen's orcin test, and Bial's reagent† gave a dull red-brown colour and formed a precipitate, but no green tint was observed. An extract of this made with four parts of amyl alcohol and one part of ethyl alcohol showed, however, a dark band between C and D when it was examined with the spectroscope. The solution showed no gas formation or diminution of its reducing powers after being incubated with brewer's yeast for 24 hours at 37° C.

These results indicated that the fluid prepared in the manner described from the urine of patients having the clinical signs and symptoms of pancreatitis contained an unfermentable sugar giving the reactions of a pentose. A careful examination of the urine in these cases, and also in over 500 others that have given a positive "pancreatic" reaction, failed to reveal any free sugar to which the reaction might be due, and it was therefore evident that the pentose was not free as such in the urine, but was derived from some antecedent substance in the course of the reaction. Numerous attempts have been made to isolate this and determine its nature, but so far without success. It does not appear to be precipitated by alcohol, ether, ammonium sulphate, magnesium sulphate, lead acetate, mercuric chloride, or calcium chloride. Treatment of the urine with benzoyl chloride and sodium hydrate has also given negative results.

Beside the case already mentioned, in which a detailed examination of the pancreas was made, it has been possible to investigate the condition of the gland after death in 26 others in which the urine had been examined during life. In 9, where no reaction had been obtained, it appeared to be normal both macroscopically and microscopically. In 13 there was evidence of chronic inflammation: these had all given a positive reaction. Four proved to be cases of cancer of the pancreas, 2 of these had given a positive reaction and 2 a negative result. Of the 13 that showed chronic pancreatitis, 12 were of the typical interlobular type, and 1 was in an early stage of inflammation with no marked increase of fibrous tissue, but an interlobular and intercellular infiltration of small round cells and granular changes in the acinar cells.

The occurrence of this reaction in the urine of patients who presented the

* Mullikin, 'Identification of Pure Organic Compounds,' 1904, p. 33.

† 'Deutsch. Med. Woch.,' 1902 and 1903.

clinical symptoms of pancreatitis, and its absence in those whose pancreas was not apparently diseased, together with the *post-mortem* evidence I had been able to obtain, suggested that it was due either to some degenerative change in the tissues of the pancreas or to a disturbance of metabolism set up by disease of the gland. To decide this question, and also to obtain confirmation of the dependence of the reaction upon disease of the pancreas, I arranged a series of experiments upon animals, the surgical part of which was kindly carried out for me by Mr. H. C. G. Semon, in the Pathological Department of the University of Freiburg. A detailed account of these experiments will be given subsequently, and I shall now only deal with those points that bear upon the questions at issue.

The urine from the dogs employed for the experiments was drawn by catheter both before and after the operations. Each specimen was shaken with a few drops of chloroform, sealed in a glass vessel, marked with a distinguishing number and letter for subsequent reference, and despatched to me in London.

The normal urine, taken before operation, gave no reaction in any of the dogs examined.

(I) A sub-acute pancreatitis was set up in the first dog by injecting a very small quantity of turpentine, less than 1 c.c., into the pancreatic duct. A specimen of the urine withdrawn 16 hours after the operation gave an exceedingly well-marked reaction, the whole bulk of the fluid being filled with a light yellow flocculent precipitate, which on microscopical examination was found to consist of long fine crystals that dissolved in 33-per-cent. sulphuric acid in 5 to 10 seconds. Filtered off, and purified by re-crystallisation from 10-per-cent. alcohol, it was found to melt at 178° to 180° C. A second specimen taken 24 hours after the operation gave a similar, but less marked, reaction.

Three days later a much larger dose of turpentine (1·5 c.c.) was injected, but by mistake this was introduced into the common bile duct instead of the pancreatic duct. The urine withdrawn six hours after this operation contained a large amount of urobilin, but gave *no* "pancreatic" reaction. Two other samples taken at the end of 18 and 24 hours respectively after the operation also yielded no osazone crystals.

A small piece of the pancreas was excised at the time of the second operation. Microscopical examination of this showed no small celled infiltration or over-growth of connective tissue, but the nuclei of the acinar cells were indistinct and the protoplasm was highly vacuolated. When the animal was killed 48 hours later examination of the pancreas revealed no pathological changes.

(II) In a second dog chronic pancreatitis was induced by passing a silk thread from the duodenum along the duct of Wirsung and leaving the loose end hanging free in the intestine.* The urine withdrawn three days after the operation yielded crowds of long fine yellow crystals, soluble in 33-per-cent. sulphuric acid in 5 to 10 seconds, and which melted at 178° to 180° C. A second specimen taken one week after the operation showed many typical crystals. A third sample obtained two weeks after the operation gave some crystals, but the reaction was not as well marked as that given by the preceding specimens. A fourth sample obtained one week later still gave a fairly well-marked reaction.

At the end of the next week the pancreas and part of the duodenum were removed and the cut ends of the intestine united by a Murphy button. Examination of the excised portions showed that the thread was still in position in the pancreatic duct and hanging into the duodenum. The whole pancreas, but more particularly the head of the gland, was thickened and felt heavier than normal. Microscopical examination of sections cut from various parts showed a marked over-growth of the interlobular connective tissue, especially in the neighbourhood of the duct of Wirsung and its larger tributaries. The epithelium of the duct was detached and in places lay loose in the lumen. The periphery of the gland was not so markedly affected, although here, too, there appeared to be some increase of fibrous tissue and a few round cells were seen in and around the ducts.

Immediately after the second operation the bladder was emptied by catheter. A specimen of urine withdrawn 15 hours later was found to contain 1·8 per cent. of reducing sugar, as estimated by Bang's method; 1·6 per cent. of fermentable sugar, estimated by Lohenstein's saccharometer, and the polariscope showed 1·2 per cent. of dextro-rotatory sugar. Treatment with phenylhydrazin gave a dense precipitate of coarse greenish-yellow crystals that were insoluble in 33-per-cent. sulphuric acid in five minutes, and on re-crystallisation from 70-per-cent. alcohol melted at 204° to 205° C., thus corresponding to dextrosazone. Forty-five cubic centimetres of the filtered urine were boiled with 3 c.c. of hydrochloric acid for 10 minutes, the excess of acid neutralised with lead carbonate, and the glycuronic acid removed by shaking with tri-basic lead acetate. The lead in the filtrate was then removed by treatment with a stream of sulphuretted hydrogen and subsequent filtration. After being heated to drive off the excess of sulphuretted hydrogen, the filtrate was cooled and mixed with half its bulk of distilled water. Yeast was then added, and the mixture incubated at

* *Cf.* Carnot, 'Gilbert et Thoinot, Traite de Médicine et de Thérapeutique,' 1908, fasc. 20, p. 238.

37° C. for 18 hours, when, as it was found that a control specimen no longer gave a reaction for sugar, it was cooled, filtered, and the filtrate treated with phenylhydrazin. Examination 24 hours later showed no crystalline deposit, either to the naked eye or microscopically. The animal was found dead three days after the operation, and *post mortem* no trace of pancreatic tissue could be discovered.

(III) The pancreas of a third dog was extirpated on September 9. Two days later it died from gangrene of the duodenum. A specimen of urine was, however, obtained before death, and 18 hours after the operation. This was found to contain 3·9 per cent. of reducing sugar (Bang), and 3·8 per cent. on fermentation (Lohenstein). Examination with the polariscope gave 3·2 per cent. of dextro-rotatory sugar. The osazone crystals melted at 204°—205° C., and were insoluble in 33-per-cent. sulphuric acid in five minutes. A specimen of the urine examined by the same method as that just described gave no "pancreatic" reaction after the fermentable sugar had been removed, there being no crystalline deposit on macroscopical or microscopical examination of a preparation left undisturbed for 24 hours.

The results of the examination of the samples from Dog I and of the specimens obtained from Dog II after the first operation agree with and confirm my experience in the human subject. They show that while normal urine gives no "pancreatic" reaction, specimens from animals in which pancreatitis has been set up, either by a chemical irritant, such as turpentine, or bacterial infection and partial blocking of the pancreatic duct, give a characteristic reaction. The accidental injection of turpentine into the common bile duct of Dog I served to prove that the reaction first obtained was not due to the turpentine itself or to the manipulation of the organs in the course of the operation, for, although considerably larger than the first dose which was injected into the pancreatic duct, it gave rise to no urinary "pancreatic" reaction. The pathological changes found in the pancreas of Dog II, when it was removed at operation, agree with those described by Carnot as being present in similar experiments performed by him, and correspond to the chronic interlobular pancreatitis met with in man as the result of an infection of the pancreatic ducts.

The results of the examination of the urines of Dogs II and III, after total extirpation of the pancreas, tend to show that the changes which give rise to the "pancreatic" reaction in the urine are dependent upon the presence of the pancreas, and are probably to be referred to changes in the gland itself and not to disturbances of metabolism in other tissues brought about by interference with or perversion of its functions, for Dog III gave

no reaction after the operation, and in Dog II the reaction which had been obtained on four occasions during the preceding three weeks disappeared after removal of the pancreas.

It may be objected that the modification of the procedure made necessary by the presence of the fermentable sugar interfered with the reliability test, but that this is not the case has been shown by the results obtained on examining the urines from several patients suffering from glycosuria associated with disease of the pancreas by the same method. One in particular demonstrated this very clearly, and also showed how an exceedingly well-marked reaction may diminish in intensity as destruction of the pancreas progresses, and finally disappears when advanced glycosuria has been established. The patient was first seen in December, 1906; there was then an abdominal tumour which was suspected to be pancreatic, but an examination of the urine gave no "pancreatic" reaction, and there was also at that time no sugar. An exploratory examination was performed by Mr. Mayo Robson, and a growth was found in the first part of the duodenum, but quite free from the pancreas. On January 18, a second specimen of urine was examined and found to be free from sugar, but it gave a well-marked "pancreatic" reaction, suggesting that the pancreas was then involved in the disease. At the request of the patient's friends the abdomen was re-opened a few days later and it was then found that the growth had invaded the pancreas as had been suspected. In the early part of May, 1906, examination of the urine showed 5·25 per cent. of sugar and a modified "pancreatic" reaction gave many fine crystals soluble in 33-per-cent. sulphuric acid in 5 to 10 seconds. A month later the sugar had increased to 7 per cent., and a much less marked "pancreatic" reaction was obtained. In July the urine contained 7·25 per cent. of sugar and the "pancreatic" reaction gave only a few crystals. In August, 7·5 per cent. of sugar was present, and no crystals were found on carrying out the modified "pancreatic" test. In October the urine contained 9·5 per cent. of sugar and the "pancreatic" reaction was negative. The patient died on November 5.

The indications afforded by the experimental, pathological, and clinical evidence so far obtained all point to the so-called "pancreatic" reaction in the urine being due to active degenerative changes in the pancreas, and, so far as I have been able to determine, to these alone. The fact that the sugar giving rise to the reaction is apparently a pentose suggests that it is probably contained in a derivative of the pancreas nucleo-protein which passes into the blood as a result of the degeneration of the gland cells. In view of the constant presence of a pentose in the nuclei of the cells of other organs and tissues it might be thought that if this were true, degeneration in these

would also furnish a pentose-yielding substance which might pass into the urine, but this does not appear to be the case. The reasons why the pancreas is probably more liable to yield a pentose complex that can be split up and so recognised in the urine are two: first, according to Gründ,* the percentage of pentose in the dry weight of the pancreas is nearly five times as great as in any other organ of the body (pancreas 2·48 per cent., liver 0·56 per cent., thymus 0·56 per cent., kidney 0·49 per cent., muscle 0·11 per cent.); second, the pentose contained in the nucleo-protein of the pancreas and thymus is said to be more loosely combined and more readily set free than the corresponding sugar in other tissues.† With regard to the first point, however, the relative bulks of the organs have to be taken into account, and it is conceivable that if the whole of an organ, such as the liver, were simultaneously involved in some degenerative change, it might yield as much or more pentose-containing substance as the pancreas under similar conditions.

Only a small part of the field of research opened up by these investigations has as yet been touched upon. It is hoped that by further experiment it may be possible to isolate the mother-substance giving rise to the pentose obtained from the urine in cases of pancreatitis, and also that a fresh series of animal experiments may furnish information as to the chemical changes in the body associated with diseases of the pancreas that precede and lead up to the disturbances of internal metabolism that give rise to diabetes.

* Hoppe-Seyler's ' Zei t. f. hysiol. Chem.,' vol. 35, p. 111.

† Blumenthal, "Dis. of Metabolism," v. Noorden's 'Clin. Med.,' 1906, p. 262.